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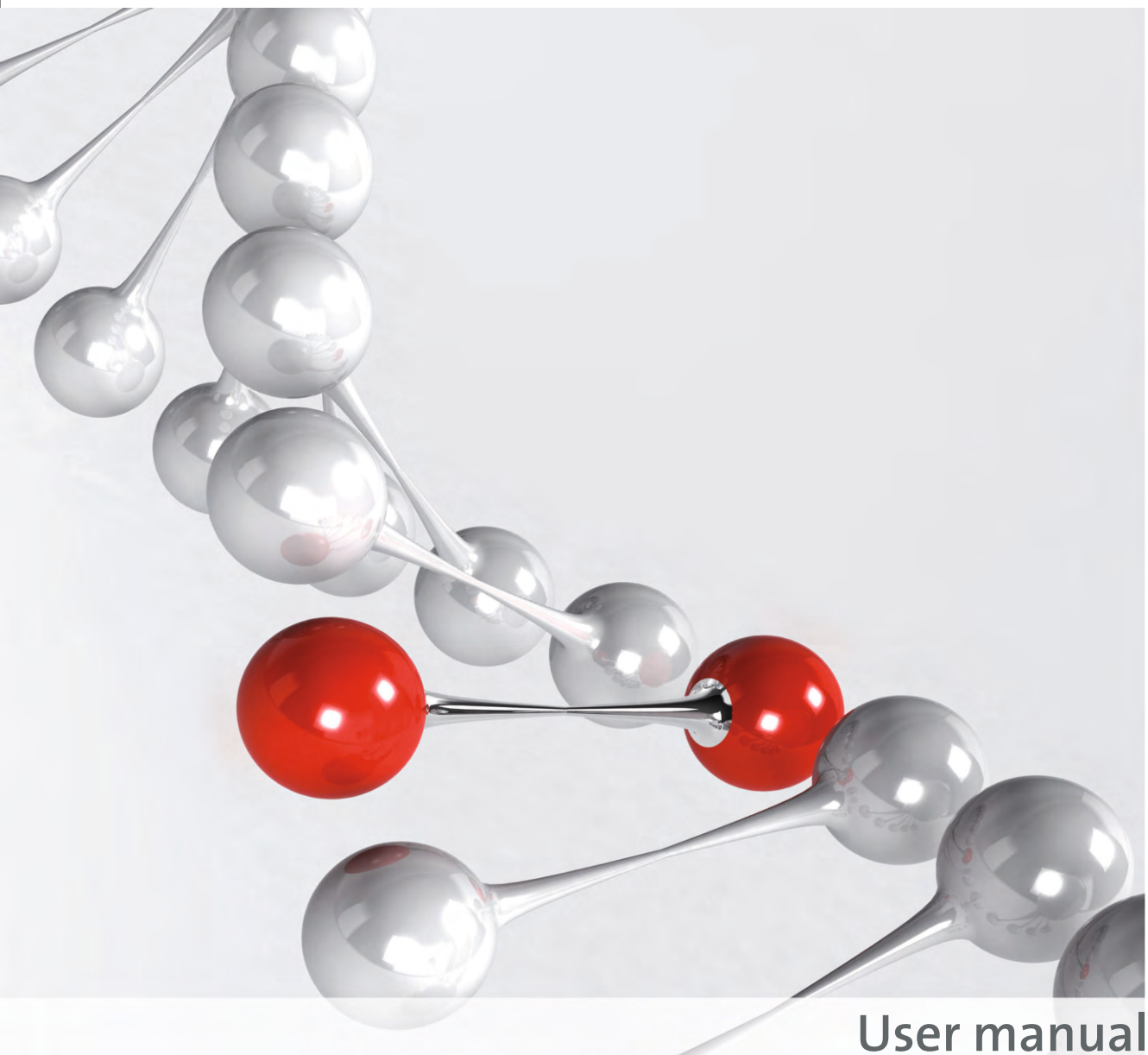

User manual
InviMag® Blood DNA Mini Kit/ KF96

for use on KingFisher® 96 and KingFisher® Flex, Thermo Fisher

for automated purification of total DNA from up to 200 µl of whole blood samples, buffy coat, non mammalian blood, bone marrow and swabs with magnetic beads

CE

IVD

REF 7431300x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Blood DNA Mini Kit /KF96

The **InviMag® Blood DNA Mini Kit /KF96** combines the advantages of the innovative InviMag® technology with easy handling of magnetic particles in combination with KingFisher instruments for an efficient and reliable isolation of genomic DNA from blood in a high purity.

The **InviMag® Blood DNA Mini Kit /KF96** is the ideal tool for a semi-automated isolation and purification of DNA (genomic and mitochondrial) from 200 µl whole blood samples (stabilized with EDTA, citrate but ***not*** heparin), buffy coat, non-mammalian blood, cerebrospinal fluid (CSF), bone marrow, and swabs in a 96 well format. The kit is designed for use on KF96 and/or KFflex96 workstations from Thermo Scientific. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Blood DNA Mini Kit /KF96** was intensely tested and validated.

The DNA-binding magnetic particles are characterized by a high surface area, uniform size distribution, good suspension stability and, therefore are highly suitable for high-throughput processing.

Due to the high purity, the isolated DNA is ready-to-use for *in vitro* diagnostic analysis in a broad panel of downstream applications or can alternatively be stored at -20°C for subsequent use.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissues, blood cards, dried blood stains, urine nor from stool samples, bacteria, fungi, parasites, total RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Trademarks: InviMag®, Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit contents of InviMag® Blood DNA Mini Kit /KF96

Store the **MAP Solution B** at 2-8°C Store dissolved **Proteinase K** at -20°C!
Store all other kit components at room temperature (RT)!

	1x 96 extractions	**5x 96 extractions**
Catalogue No.	7431300100	7431300200
Lysis Buffer HLT	30 ml	120 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Proteinase K (working solution)	2 x 1.1 ml	10.5 ml
MAP Solution B	4 x 1.1 ml	2 x 10.5 ml
Wash Buffer HLT	90 ml (final volume 150 ml)	360 ml (final volume 600 ml)
Wash Buffer M	30 ml ((final volume 120 ml)	150 ml (final volume 600 ml)
Wash Buffer II	45 ml (final volume 150 ml)	180 ml (final volume 600 ml)
Elution Buffer M	15 ml	60 ml
2.0 ml Deep Well Plate	4 pieces	20 pieces
KF 96 Tip Comb for DW magnets	1 piece	5 pieces
200 µl Elution Plate*	2 pieces	10 pieces
1.5 ml Receiver Tubes	2 x 50 pieces	10 x 50 pieces
Sealing Foils	2 pieces	10 pieces
Manual	1	1
Initial steps	Add 60 ml of 99.7% isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly Add 1.1 ml of distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving and store at -20°C! Fill 30 ml **Isopropanol** (molecular biologic grade) into the empty bottle	Add 240 ml of 99.7% isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly Add 450 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly Add 10.5 ml of distilled water to each **Proteinase K** tube mix thoroughly until completely dissolving and store at -20°C! Fill 120 ml **Isopropanol** (molecular biologic grade) into the empty bottle

* Elution Plates and Tip Plates are identical. Use one provided Elution Plate as a Tip Plate.

Kit contents of InviMag® Blood DNA Mini Kit /KF96 w/o plastic

Store the **MAP Solution B** at 2-8°C! Store dissolved **Proteinase K** at -20°C!
Store all other kit components at room temperature (RT)!

	1 x 96 extractions	**5 x 96 extractions**
Catalogue No.	7431300150	7431300250
Lysis Buffer HLT	30 ml	120 ml
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 30 ml)	empty bottle (final volume 120 ml)
Proteinase K (working solution)	2 x 1.1 ml	10.5 ml
MAP Solution B	4 x 1.1 ml	2 x 10.5 ml
Wash Buffer HLT	90 ml (final volume 150 ml)	360 ml (final volume 600 ml)
Wash Buffer M	30 ml ((final volume 120 ml)	150 ml (final volume 600 ml)
Wash Buffer II	45 ml (final volume 150 ml)	180 ml (final volume 600 ml)
Elution Buffer M	15 ml	60 ml
1.5 ml Receiver Tubes	2 x 50 pieces	10 x 50 pieces
Sealing Foils	2 pieces	10 pieces
Manual	1	1
Initial steps	Add 60 ml of 99.7% Isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly Add 1.1 ml of distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving and store at -20°C! Fill 30 ml **Isopropanol** (molecular biologic grade) into the empty bottle	Add 240 ml of 99.7% Isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly Add 450 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly Add 10.5 ml of distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving and store at -20°C! Fill 120 ml **Isopropanol** (molecular biologic grade) into the empty bottle
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	4 pieces	20 pieces
KF 96 Tip Comb for DW magnets	1 piece	5 pieces
200 µl Elution Plate*	2 pieces	10 pieces

* Elution Plates and Tip Plates are identical. Use one provided Elution Plate as a Tip Plate.

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **InviMag® Blood DNA Mini Kit /KF96**, except dissolved **Proteinase K** and **MAP Solution B** should be stored at room temperature and are stable for at least 12 months at these conditions.

Proteinase K: Dissolved Proteinase K must be stored at -20°C. Dividing the Proteinase K into aliquots and storage at -20°C is recommended. Avoid multiple freezing and thawing cycles because this will lead to decreased enzymatic activity.

MAP Solution B: The magnetic particles should be stored at 2-8°C.

Wash Buffer HLT, Wash Buffer M, Wash Buffer II, Binding Solution: Wash Buffers and Binding Solution charged with either ethanol or isopropanol should be stored well sealed at room temperature*. If any precipitates are visible within the provided solutions solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Blood DNA Mini Kit/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Blood DNA Mini Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Blood DNA Mini Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Blood DNA Mini Kit/ KF96** is designed for semi-automated extraction and purification of total (genomic and mitochondrial) DNA from 8-96 whole blood or blood related samples using magnetic beads and the KF96 or KFlex96 instrument. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA from fresh or frozen whole blood samples, buffy coats, non-mammalian bloods, cerebrospinal fluids (CSF), bone marrow, and swabs. For reproducible and high yields appropriate sample storage is essential (see "Sampling and storage of the starting material", page 10). Common blood collection tubes (not provided) and anticoagulants (EDTA, citrate but ***not heparin***) can be used to assemble a set of blood samples. All utilities (reagents and plastic ware) necessary for preparation of total DNA are provided by the **InviMag® Blood DNA Mini Kit/ KF96** in different package sizes.

The procedure of the **InviMag® Blood DNA Mini Kit/ KF96** is optimized for the isolation of total DNA from up to 200 µl starting material. For samples of a smaller volume than 200 µl please adjust to a total sample volume of 200 µl with 1x PBS prior to the start of an isolation protocol.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, blood cards, dried blood stains, urine nor from stool sample, bacteria, fungi, parasites, or the purification of total RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Blood DNA Mini Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste has be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Blood DNA Mini Kit/ KF96** to which they apply, are listed below as follows:.

Wash Buffer I contains guanidine thiocyanate which is an irritant.

Lysis Buffer HLT, Wash Buffer HLT

warning

contains guanidine-hydrochloride;

H302-315-319, P280-305+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the InviMag® Blood DNA Mini Kit /KF96

The **InviMag® Blood DNA Mini Kit /KF96** procedure is the ideal tool for an efficient DNA extraction and purification from fresh or frozen whole blood samples, non-mammalian blood, buffy coat, CST, bone marrow, and swabs in a convenient 96 well format using magnetic beads and the KF96/KFflex96 instrument.

Starting Material	Yield	Run Time	Purity
1-200 µl fresh or frozen human or other mammalian whole blood 1-200 µl cerebrospinal fluid 1–30 µl buffy coat 1–25 µl fresh, frozen or old non mammalian blood 1–20 µl bone marrow swabs up to 200 µl rinsed liquid from swab	3–8 µg, depends on the blood sample (kind, storage and source)	about 54 min	A_{260}:A_{280}: 1.8-2.2

The semi-automated DNA isolation process is based on the interaction of nucleic acids with coated magnetic particles at adapted buffer conditions. The KingFisher instrument performs all steps of the DNA purification procedure automatically without any user intervention. The procedure requires only minimal interaction by the user, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by the automated purification process.

The KingFisher® instrument uses magnetic rods to transport the DNA-binding magnetic particles through the various purification phases such as binding, washing, drying and elution. The volume of buffers and other liquids required for isolation is reduced to a minimum. Eliminating most of the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific cell lysis on the instrument, using the **Lysis Buffer HLT** and **Proteinase K**, optimal binding conditions are adjusted by addition of **Binding Solution.** The released DNA binds to the simultaneously added **MAP Solution B** (magnetic particles) and is separated from solution by magnetic rods controlled by the KingFisher machine. Subsequent to three washing steps using **Wash Buffer HLT, Wash Buffer M** and **Wash Buffer II**, the DNA is finally eluted in **Elution Buffer M**.

Due to the high purity, the eluted total (genomic and mitochondrial) DNA is ready-to-use in a broad panel of downstream applications:

- PCR*, Real-time PCR, PCR, qPCR
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot

The **InviMag® Blood DNA Mini Kit /KF96** is supplied with a comprehensive manual describing four protocols (page 14-15) for DNA purification from different sample sources. For the semi-automated isolation of genomic DNA from 200 µl blood using magnetic particles in a single well format for up to 15 samples per run, STRATEC Molecular offers the **InviMag® Blood DNA Mini Kit/ KFmL** for use on a KingFisher mL instrument and for 1 ml whole blood the **InviMag® Blood DNA Midi Kit /KFflex24.**

* The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

For the isolation of DNA from a single blood sample STRATEC Molecular offers the **Invisorb® Spin Blood Mini Kit** or for 8–96 samples the **Invisorb® DNA Blood Mini HTS 96 Kits** for use on a centrifuge or robotic station (see “Ordering information”, page 24).

For further information please contact +49 (0) 30 9489 2901 or 2910 in Germany and +49 (0) 30 9489 2907 from foreign countries or ask your local distributor.

Product validation

PCR inhibitor and cross contamination test

To maximize the detection of any potential contamination event, positive and no template controls (NTCs) were arranged in alternating wells (in a “chessboard” pattern ***Fig. 1***). Out of those samples ***Fig. 2*** shows a real-time PCR run of the extracted DNA. PCRs were done with a GAPDH Primer set in an in-house SyBr Green assay on a Corbett Rotor Gene 3000.

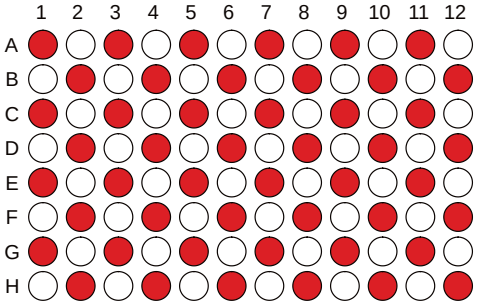


Fig. 1 **‘Chessboard Pattern’** utilized for the cross contamination analysis test. Samples (red) and NTC (white) arranged in alternating wells.

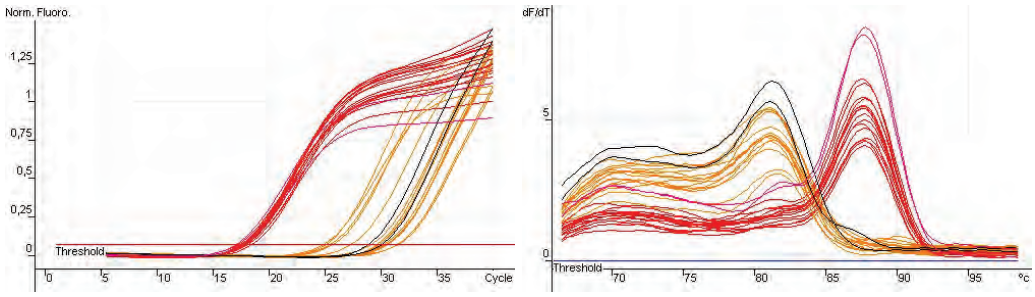
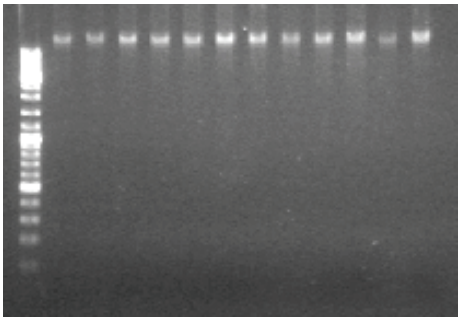


Fig. 2 Real-time PCR results from samples (red) and no samples controls (yellow) arranged in Chessboard. NTC (black) and PTC (pink) are also shown.

Reproducibility



DNA was isolated from 200 µl transfusion blood

The ratio $A_{260/280}$ is between 1.94 ± 0.06

The yield is about (3.5±0.3) µg.

Sampling and storage of starting material

For reproducible and high yields appropriate sample storage is essential. Yields may be varying from sample to sample depending on factors such as health of the donor, sample age, kind of sample, transport and storage conditions.

Blood and buffy coat:
Best results are obtained using fresh blood samples. Mammalian blood samples (stabilized with EDTA or Citrate) can be stored at room temperature for 2-3 hours, for short-time storage (up to 24 h) samples may be stored at 4°C. For long-term storage, we recommend freezing samples at -20°C or -80°C. Multiple thawing and freezing cycles before isolating the DNA should be avoided. If cryoprecipitates (formed during thawing of frozen samples) are visible avoid aspirating them. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (except heparin) can be used to collect blood samples for the **InviMag® Blood DNA Mini Kit /KF96** procedure.

Buffy coat is a whole blood fraction of enriched leukocyte cells. To prepare and extract a buffy coat layer the following procedure is recommended: The use of a whole blood sample (anticoagulants: EDTA, citrate, ***not*** heparin) with a sedimented cellular fraction from staying overnight at 4°C is recommended. The resulting bright mid-section overlaid by the clear plasma is buffy coat containing concentrated leukocytes that can be easily distinguished from the erythrocytes in the bottom layer. An enrichment factor of 10 is expected from such a procedure. Due to the enriched leukocyte content be aware to avoid overloading the system.

CSF (cerebrospinal fluid) and bone marrow:
Best results are obtained with fresh material that can be stored for 2-3 h at 4°C for short-term storage. For long-term storage freezing at -20°C is recommended. Dried samples have to be stored at 4°C in a dry surrounding.

Swabs:
The protocol works with fresh prepared swabs as well as with dried swabs. Please note, that dried swab samples are often characterized by isolation of apoptotic DNA (visible on agarose gel as a typical apoptotic DNA band pattern).The protocol has not been validated for isolation of DNA from swabs which are stored in special storage buffers from other providers.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and procedure

The **InviMag® Blood DNA Mini Kit /KF96** procedure comprises following steps:

- lysis of blood cells and protein digestion
- binding the genomic DNA to magnetic beads
- washing of the bead bound DNA and elimination of ethanol
- elution of genomic DNA

After lysis, the DNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three washing steps and highly purified DNA is eluted in Elution Buffer D.

This manual contains 4 protocols (see page 14-15).

Lysis

Samples with a volume lower than 200 µl should be adjusted to 200 µl using 1x PBS or distilled water before starting the protocol. For optimal results, samples must be equilibrated at room temperature before lysis.

Samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K**. In case of large number of samples the preparation of a master mixture with a volume 5% greater than that required for the processing of all samples is recommended.

Binding of the genomic DNA

After adding **Binding Solution** and **MAP Solution B**, the DNA is bound to the surface of the magnetic beads.

Removing residual contaminants

Contaminants are efficiently removing using **Wash Buffer HLT, Wash Buffer M** and **Wash Buffer II**, while the DNA remains bound to the magnetic beads.

Elution

The DNA is eluted in **Elution Buffer M** and is ready-to-use in different subsequent downstream applications like:

- PCR amplification
- digestion with restriction enzymes
- Southern hybridizations
- HLA typing, etc.

Yield and Quality

The amount of purified DNA in the **InviMag® Blood DNA Mini Kit /KF96** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage, and age.

Typically, a 200 µl sample of whole blood cells (samples with elevated white blood cell (WBC counts), ranging from $3x10^6$ to $1x10^7$ cells/ml) from a healthy individual will yield 3-8 µg of DNA. If the whole blood sample is mixed with anticoagulant containing buffer solutions the overall leukocyte concentration decreases and the yield of the DNA extraction procedure is reduced.

Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed accordingly to manufacturers' specifications.

Important notes

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between different liquid transfers. To avoid cross-contaminations, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components from different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, equilibrate all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

Lysis Buffer HLT and **Elution Buffer M** are ready-to-use.

Prepare all other reagents as indicated below:

1 x 96 DNA-extractions:
Add 60 ml of 99.7% isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly
Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly
Add 90 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly
Add 1.1 ml of distilled water to each Proteinase K tube, mix thoroughly until completely dissolving and store at -20°C!
Fill 30 ml **Isopropanol** (molecular biologic grade) into the empty bottle
5 x 96 DNA-extractions:
Add 240 ml of 99.7% isopropanol to each bottle **Wash Buffer HLT** and mix thoroughly
Add 420 ml of 96-100% ethanol to the bottle **Wash Buffer II** and mix thoroughly
Add 450 ml of 96-100% ethanol to the bottle **Wash Buffer M** and mix thoroughly
Add 10.5 ml of distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving and store at -20°C!
Fill 120 ml **Isopropanol** (molecular biologic grade) into the empty bottle

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 ml or 2.0 ml)
- dd-H_2O
- 96-100% ethanol
- 99.7% isopropanol

*The **InviMag® Blood DNA Mini Kit /KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

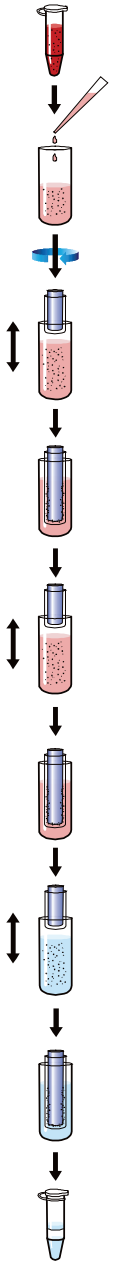
Scheme of the InviMag® Blood DNA Mini Kit /KF96

Please read protocols prior the start of the preparation carefully

Transfer 200 µl **Lysis Buffer HLT** and 200 µl **sample** into a cavity of a 2 ml Deep Well Plate (refers as "Lysis Plate") and 20 µl Proteinase K.

Prefill all remaining plates with required buffers and appropriate volumes.

Tip Plate:	Insert the KF96 Tip Comb for DW magnets on a Tip Plate*
Lysis Plate:	Add 200 µl **Lysis Buffer HLT**, 200 µl **sampl**e, 20 µ **Proteinase K**
Washing Plate_1:	Add 800 µl **Wash Buffer HLT** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add 900 µl **Wash Buffer M** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add 900 µl **Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer M** to the Elution Plate (same size as Tip Plate)



Please read the protocols carefully prior to the start of the preparation procedure!

The following steps are performed on the KingFisher instrument:

Lysis of the sample

After lysis, a pause step occurs and 230 µl **Binding Solution** and 40 µl **MAP Solution B** have to be added

Nucleic acids bind to magnetic particles

Washing of the particle fixed nucleic acids

Magnetic separation

Elution of nucleic acids

Magnetic Separation

Pure nucleic acids

* Elution Plates and Tip Plates are identically. Use one provided Elution Plate as a Tip Plate.

Lysis Procedures

Protocol 1: Isolation of genomic DNA from up to 200 µl of whole blood / up to 30 µl of buffy coat

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *Samples with a smaller volume than 200 µl must be adjusted to a final volume of 200 µl using either 1x PBS or distilled water.*

1. Transfer 200 µl of whole blood or 30 µl buffy coat into a free cavity of the Lysis Plate and add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** to each sample containing cavities.

2. Proceed with prefilling of the plates and preparation of the instrument (see "Starting a Run", page 16)

Protocol 2: Isolation of genomic DNA from up to 30 µl of non-mammalian blood

Please read the instructions carefully and conduct the prepared procedure.

Important note: Bird (e. g. chicken) or fish blood contains nucleated erythrocytes. Therefore, only 10-15 µl of starting material should be used for isolation.

1. Transfer max. 30 µl of non-mammalian blood (***not*** *heparin stabilized*) into the cavities of the Lysis Plate. Adjust the sample volume to 200 µl with 1x PBS or distilled water.

2. Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** to sample containing cavities of the Lysis Plate.

3. Proceed with prefilling of the plates and preparation of the instrument (see "Starting a Run", page 16)

Protocol 3: Isolation of genomic DNA from CSF and bone marrow

Please read the instructions carefully and conduct the prepared procedure.

Preparation of the starting material:

Fresh material:

- 1–200 µl fresh cerebrospinal fluid
- 1–20 µl bone marrow

Dried material (for example on hematological slides):

- Moisten the dried material with a drop of PBS.
- Add 180 µl PBS to a 1.5 ml reaction tube (not provided) and scrape the cytological material into the tube using the edge of a clean slide.
- Dissolve the resulting sludge by pipetting up and down several times.

1. Transfer the starting material into free cavities of the Lysis Plate. Adjust the sample volume to 200 µl with 1x PBS or distilled water.

2. Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** to sample containing cavities of the Lysis Plate.

3. Proceed with prefilling of the plates and preparation of the instrument (see "Starting a Run", page 16)

Protocol 4: Isolation of genomic DNA from swabs or rinsed liquid from swabs

Please read the instructions carefully and conduct the prepared procedure.

Dried swabs:

If the swab is delivered without transportation media, rinse the swab in a 1.5 ml reaction tube filled with 200-300 µl cooled water or 1x PBS. Mix for several minutes by shaking and continue with step 1 (see below).

Rinsed swabs:

1. Squeeze out the swab inside the wall of the transportation tube and discard it.

2. Transfer 200 µl from the transportation media /jetting liquid into a 1.5 ml reaction tube (not provided). If the sample volume is lower than 200 µl, adjust with 1x PBS or distilled water.

3. Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K** to each sample containing cavity of the Lysis Plate.

4. Proceed with prefilling of the plates and preparation of the instrument (see “Starting a Run”, page 16)

Starting a run on a KF96 / KFflex96 instrument

Important: *For working with the KingFisher instruments please carefully read the manufacturer's instructions before use!*

1. Turn on the KF96 or KFflex96 instrument

Note: *Use one provided Elution Plate as Tip Plate. These plates are identical.*

2. Prefill all Deep Well Plates with the appropriate buffers and volumes as indicated below.

3. **Tip Plate**: Place the provided Tip Comb for DW magnets on a 200 µl Elution Plate (these are identical).

4. **Lysis Plate:** Add 200 µl **sample**, 20 µl **Proteinase K** and 200 µl **Lysis Buffer HLT**. After lysis, a pause step occurs and 230 µl **Binding Solution** and 40 µl **MAP Solution B** have to be added to each sample containing cavity

Washing plate_1: Add 800 µl **Wash Buffer HLT**

Washing plate_2: Add 900 µl **Wash Buffer M**

Washing plate_3: Add 900 µl **Wash Buffer II**

Elution Plate: Add 100 µl **Elution Buffer M**

Important: *Mix the bottle with the **MAP Solution B** by vigorously vortexing!*

5. Choose either the KF96 protocol **"InviMag_Blood_KF96"** or the KFflex96 protocol **"InviMag_Blood_KFflex96"** depending on the used instrument and press the "START" button.
6. Insert all prefilled plates into the instrument by following the specifications printed on the display and confirm every plate loading step by pressing the "START" button.
7. After all prefilled plates have been loaded press the "START" button to initialize the assay file. The run will take approximately 54 min.

Important: *After lysis, a pause step occurs and 230 µl **Binding Solution** and 40 µl **MAP Solution B** have to be added to each sample containing cavity of the Binding Plate. To achieve that, the plate is transported to the initial loading position. After adding both reagents, reinsert the plate into the instrument and confirm this step by pressing the "START" button again. The instrument will continue with the extraction without any further user interaction. Watch out that the orientation of the reinserted plate is correct.*

The following extraction steps run automatically on the KingFisher instrument:

Lysis of the blood cells

Automatically sample mixing for 15 min at elevated temperature.

Adjustment of Binding condition

Magnetic Beads (MAP Solution B) and Binding Solution are added to the lysed sample mixture

Binding of the DNA

Automatically sample mixing for 5 min. MAP Solution B separation. Moving of the MAP Solution B into the Washing Plate_1.

First Washing

Automatically sample mixing for 3 min. MAP Solution B separation. Moving of the MAP Solution B into the Washing Plate_2.

Second Washing

Automatically sample mixing for 2 min. MAP Solution B separation. Moving of the MAP Solution B into the Washing Plate_3.

Third Washing and Drying

Automatically sample mixing for 90 s. MAP Solution B separation. Drying the MAP Solution B outsight the plate for 5 min. Moving of the MAP Solution B into the Elution Plate.

Elution of the DNA

Incubation of the MAP Solution B into the Elution Plate for 10 min by mixing at elevated temperatures. MAP Solution B separation.

The MAP Solution B will then be automatically removed into the wells of Washing Plate_3 (disposal).

Important Note: After finishing the extraction protocol, the Elution Plate contains the extracted DNA. We recommend store the DNA at -20°C.

If the extracted DNA contains carryover of magnetic particles, transfer the DNA to a 1.5 ml reaction tube, centrifuge at maximum speed (13000 rpm) for 1 min and transfer the DNA-containing supernatant into a new tube.

For self-programming the KF96 / KFflex96 instrument

Reagent info

Tip Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Lysis Plate		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Sample	200	-	Sample
Lysis Buffer HLT	200	-	Reagent
Proteinase K	20	-	Reagent

Wash Plate 1		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer HLT	800	-	Reagent

Wash Plate 2		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer M	900	-	Reagent

Wash Plate 3		Microtiter DW 96 plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	900	-	Reagent

Elution Plate		KingFisher 96 KF plate	
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer M	100	-	Reagent

Dispensed reagents

Lysis Plate		Microtiter DW 96 plate	
Name	**Step**	**Well volume [µl]**	**Total reagent volume [µl]**
Isopropanol	Adjust Binding Condition	230	-
MAP Solution B	Adjust Binding Condition	40	-

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip Plate	
	Lysis Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release beads	Yes
	Mixing / heating:	Mixing time, speed	00:15:00, Medium
		Heating temperature [°C]	75
		Preheat	Yes
	End of step	Postmix	No
		Collect beads	No
	Adjust Binding Condition	Lysis Plate	
		Message	Add Isopropanol + MAP Sol. B
		Dispensing volume [µl]	270
	Reagent(s)	Name	Isopropanol
		Volume [µl]	230
		Name	MAP Solution B
		Volume [µl]	40
	Binding Step	Lysis Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing Step 1	Wash Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:03:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Washing Step 2	Wash Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:02:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5

	Washing Step 3	Wash Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Drying Step	Wash Plate 3	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution Step	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	65
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	10
	Bead Removal Step	Wash Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
Low amount of extracted DNA	Insufficient lysis	Increase lysis time, but prevent too long lysis time because this will decreases the yield or reduce amount of starting material
	Incomplete elution	Increase the volume of **Elution Buffer M.** Ensure that the **Elution Buffer M** is transferred to the right cavity.
	Inhomogeneous amount of beads	Mix **MAP Solution B** vigorously before use
Low concentration of extracted DNA	Too much Elution Buffer	Elute the DNA in a lower volume of **Elution Buffer M**.
	Incorrect storage of starting material	Ensure that the storage of starting material was correct. Avoid repeated thawing and freezing cycles of the sample material
	Incorrect Wash Buffers	Ensure, that the correct amount of ethanol / isopropanol is added to the **Wash Buffers** and storage is correct
Degraded DNA	Incorrect storage of starting material	Ensure that the storage of starting material was correct
	Old material	Ensure that the starting material is stored at appropriate conditions (–20°C/-80°C) avoid multiple thawing and freezing cycles of the material
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	No PCR result for genomic DNA	Due to the very gentle isolation procedure it may occur that isolated genomic DNA forms a clew. To overcome this, the first primary PCR denaturation step at 95°C should be prolonged to 5 min
	Ethanol carryover during elution	Increase drying time for removal of ethanol in the assay file
	Salt carry-over during elution	Check **Wash Buffers** for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C Ensure that the **Wash Buffers** are equilibrated at room temperature before usage
Eluted DNA is brownish colored	Small part of the magnetic particles are left in the elution	Centrifuge at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note*:* *When creating assay files for usage with KingFisher instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and/or enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure its function in various downstream applications. Damaged DNA performs poorly in applications such as Southern blotting, long-template PCR and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -80°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA at 2-8°C. Storing genomic DNA at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles. Plasmid DNA and other small circular DNAs can be stored at 2-8°C or at -20°C.

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to air dry DNA than to use a vacuum. Although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively incubate the DNA in buffer overnight at 2-8°C. Minimize vortexing of genomic DNA because this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings may cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings especially designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid or other small DNA.

Ordering information

Product	Package size	Catalogue No.
InviMag® Blood DNA Mini Kit /KF96	1 x 96 preparations	7431300100
InviMag® Blood DNA Mini Kit /KF96	5 x 96 preparations	7431300200

KingFisher 96 and consumables

KingFisher 96, Magnetic Particle Processor,100-240V,50/60Hz (including one magnetic head)	5400500
KingFisher 96 Head for Deep Well plate	24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box	97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box	97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box	97002534
KingFisher 96 KF plate (200ul) 48 plates / box	97002540
Microtiter deep well 96 plate, 50 plates/box	95040450

Related products	Package size	Catalogue No.
InviMag® Blood DNA Mini Kit/ KFmL	15 preparations	2431110100
InviMag® Blood DNA Mini Kit/ KFmL	75 preparations	2431110200
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Invisorb® Spin Blood Midi Kit	50 preparations	1031130200
Invisorb® Blood Universal Kit	500 ml	1031150200

using a centrifuge

Invisorb® DNA Blood Mini HTS 96 Kit/ C	4 x 96 preparations	1031300300
Invisorb® DNA Blood Mini HTS 96 Kit/ C	24 x 96 preparations	1031300400

using the X-tractor Gene™, Corbett Robotics

Invisorb® Blood Mini HTS 96 Kit/ X	4 x 96 preparations	7131310300
Invisorb® Blood Mini HTS 96 Kit/ X	24 x 96 preparations	7131310400

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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